D-2875

Amendments to the Specification:

The paragraph on page 4, line 10 has been amended as follows:

--As discussed in Nichols et al, *supra*, spinothalamic and spinoparabrachial neurons are involved in the ascending conduction of acute noxious stimuli. Apparently, these neurons are projection neurons and can be targeted by substance P. When a conjugate of the ribosome-inactivating protein saporin and SP was intrathecally infused into the spinal cord, the SAP-SP conjugate is stated to have specifically concentrated in the projection neurons apparently because these neurons express surface receptors for substance P (a substance P receptor can be abbreviated as "SPR"). Unfortunately, the SAP-SP targeted neurons are killed by the SAP.--

The paragraph on page 7, line 9 has been amended as follows:

--The last step of the mechanism of botulinum toxin activity appears to involve reduction of the disulfide bond joining the H and L chain. The entire toxic activity of botulinum and tetanus toxins is contained in the L chain of the holotoxin; the L chain is a zinc (Zn++) endopeptidase which selectively cleaves proteins essential for recognition and docking of neurotransmitter-containing vesicles with the cytoplasmic surface of the plasma membrane, and fusion of the vesicles with the plasma membrane. ~~tetanus~~ Tetanus neurotoxin, botulinum toxin/B/D,/F, and/G cause degradation of synaptobrevin (also called vesicle-associated membrane protein (VAMP)), a

D-2875

synaptosomal membrane protein. Most of the VAMP present at the cytosolic surface of the synaptic vesicle is removed as a result of any one of these cleavage events. Each toxin specifically cleaves a different bond.--

The paragraph on page 11, line 16 has been amended as follows:

--The tetanus neurotoxin acts mainly in the central nervous system, while botulinum neurotoxin acts at the neuromuscular junction; both act by inhibiting acetylcholine release from the axon of the affected neuron into the synapse, resulting in paralysis. The effect of intoxication on the affected neuron is long-lasting and until recently has been thought to be irreversible. The tetanus neurotoxin is known to exist in one immunologically distinct type.--

The paragraph beginning on page 13, line 25 has been amended as follows:

--Thus, the agents disclosed by Foster et al are non-specific because their targeting moieties are not known to bind to receptors specifically and to primarily localize to primary sensory afferent nerve terminals. Therefore, the targeting moieties disclosed by Foster et al. may readily bind to receptors on neuronal terminals, or neurons, that are not primary sensory afferent synaptic terminals. For example, the targeting moiety comprising nerve growth factor disclosed by Foster et al may readily bind to receptors on nerve terminals and neurons other than the receptors on the primary sensory

afferent nerve terminals, because nerve growth factor receptors are found on most neurons. As such, the clostridial neurotoxin conjugate disclosed by Foster et al may bind to one of these other neurons, for example the neurons involved in the sympathetic pathway, translocate into their cytosol, inhibit the release of their neurotransmitters, and thereby inhibiting their functions. Such random, non-specific inhibition may cause undesirable side effects during the treatment if pain.--

The paragraph on page 17, line 21 has been amended as follows:

--The agents ~~disclose~~ disclosed herein comprise a polypeptide, with a first and second amino acid sequence regions. The first region preferably includes a first domain and a second domain. Preferably, the first domain comprises a targeting moiety, and the second domain comprises the H_N. The targeting moiety is the same as described above. The H_N preferably is derived from *Clostridial botulinum* type A and is able to facilitate the transfer of the entire polypeptide, or portions of the polypeptide, preferably the second amino acid region, across an intracellular endosome membrane into the cytosol of the neuron.--

The paragraph on page 18, line 9 has been amended as follows:

--In one embodiment, recombinant techniques are used to produce the clostridial neurotoxin components of the present agents. The technique includes generating genetic constructs which have codes for clostridial neurotoxins, modified clostridial neurotoxins, or fragments thereof. The genetic

constructs are then fused with cloning vectors, such as plasmids, and are incorporated into a host cell for amplification. The expressed clostridial components can then be then isolated by conventional and known techniques.--

The paragraph on page 20, line 20 has been amended as follows:

--H_N means a fragment (about 50 kDa) derived from the H chain of a clostridial neurotoxin which is approximately equivalent to the amino end segment of the H chain, or the portion corresponding to that fragment in the intact [[in the]] H chain. It is believed to ~~contains~~ contain the portion of the natural or wild type clostridial neurotoxin involved in the translocation of the L chain across intracellular endosomal membrane.--

The paragraph on page 20, line 26 has been amended as follows:

--LH_N L-H_N means a fragment derived from a clostridial neurotoxin that contains the L chain, or a functional fragment thereof coupled to the H_N domain. It can be obtained from the intact clostridial neurotoxin by proteolysis, so as to remove or modify the H_c domain.--

The paragraph on page 21, line 17 has been amended as follows:

D-2875

--Significantly, the agents of the present invention can alleviate pain without being cytotoxic to their target neurons. Furthermore, agents within the scope of the present invention can be administered to both central nociceptive neurons and to primary sensory afferent neurons.--

D-2875

The paragraph on page 23, line 7 has been amended as follows:

--Furthermore, the clostridial neurotoxin component may ~~comprises~~ comprise only a fragment of the entire neurotoxin. For example, it is known in the art that the H_c of the neurotoxin molecule can be removed from the other segment of the H chain, the H_N, such that the H_N fragment remains disulphide linked to the L chain of the neurotoxin molecule to provide a fragment known as ~~known as~~ the LH_N. Thus, in one embodiment of the present invention the LH_N fragment of a clostridial neurotoxin is covalently coupled, using linkages which may include one or more spacer regions, to a targeting component.—

The paragraph on page 24, line 1 has been amended as follows:

--In another embodiment of the invention, the L chain of a clostridial neurotoxin, or a fragment of the L chain containing the endopeptidase activity, is linked, using linkages which may include one or more spacer regions, to a targeting moiety which can also effect the internalization of the L chain, or fragment thereof containing endopeptidase activity, into the cytoplasm of the cell.--

The paragraph on page 29, line 1 has been amended as follows:

--In one embodiment, the spacer region is made up of sugar molecules, for example, saccharides, glucose, etc. In another

D-2875

embodiment, the spacer region may be constructed from [[a]] an aliphatic chain. In another embodiment, the spacer regions may be constructed by linking together a series of amino acids, preferably glycine because they are small and are devoid of any functional group. In yet another embodiment, the spacer region may comprise one or more of the sugar molecules, aliphatic chains, and amino acids.--

The paragraph on page 29, line 29 has been amended as follows:

--The second amino acid sequence region preferably comprises the L chain. The L chain is the effective therapeutic element having biological activity because, as discussed above, once it is transferred inside the neuron it interferes with the exocytosis process of neurotransmitters. Preferably, the light chain is derived from *Clostridial botulinum* neurotoxin type A. According to another broad aspect of this invention recombinant techniques are used to produce the clostridial neurotoxin components of the agents. The technique includes steps of obtaining genetic materials from either DNA cloned from natural sources, or synthetic oligonucleotide sequences, which have codes for clostridial neurotoxin components including clostridial neurotoxins, modified clostridial neurotoxins and fragments thereof. The genetic constructs are incorporated into host cells for amplification by first fusing the genetic constructs with a cloning vectors, such as phages or plasmids. Then the cloning vectors are inserted into hosts, preferably E. coli's. Following the expressions of the recombinant genes in host cells, the resultant proteins can be isolated using

D-2875

conventional techniques. The clostridial neurotoxin components derived from the recombinant techniques can then be chemically coupled to targeting moieties. Preferably, the linkages between the clostridial components and the targeting moieties include [[an]] appropriate spacer regions.--